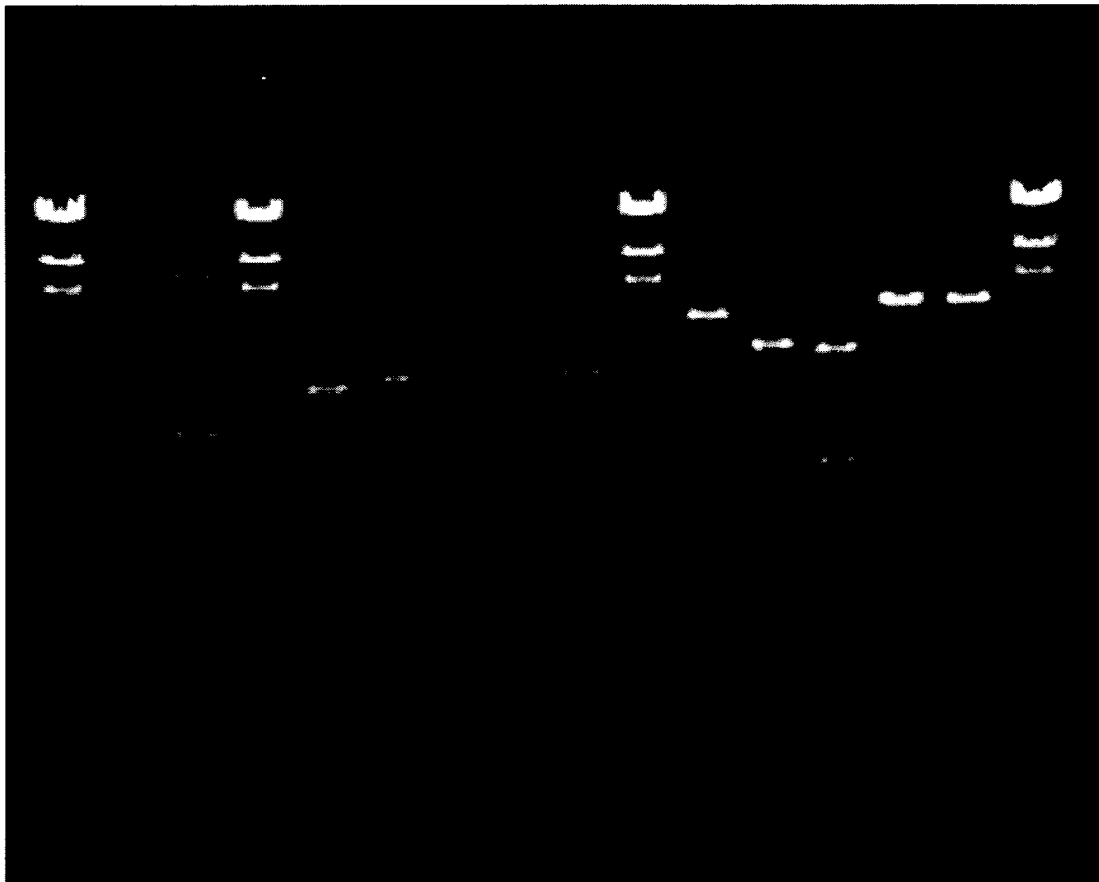


BtgZI Figure 1: Agarose gel showing BtgZI cleavage of lambda, T7, pBR322 and PhiX174 DNAs.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



BtgZI Figure 2 : Determination of the BtgZI cleavage site.

Figure 2A: Location of cleavage on 5'-CATCGC-3' strand.

A DNA (Sep-S3AI8.G10R) having a BtgZI site and a convenient primer was cut with BtgZI, yielding ends indicated by the arrows:

5'--..CGATGAGAATGCGATGTTGGTGGCCA↓AAAGCAATTATCC..-3'
(SEQ ID NO:1)

3'--..GCTACTCTTACGCTACAACCACCGGTTTTTC↑GTTAATAGG..-5'

The resulting cleaved DNA:

5'--..CGATGAGAATGCGATGTTGGTGGCCA-3' (SEQ ID NO:2)

3'--..GCTACTCTTACGCTACAACCACCGGTTTTTC-5'

The template strand for dideoxy DNA sequencing extension:

3'--..GCTACTCTTACGCTACAACCACCGGTTTTTC-5'

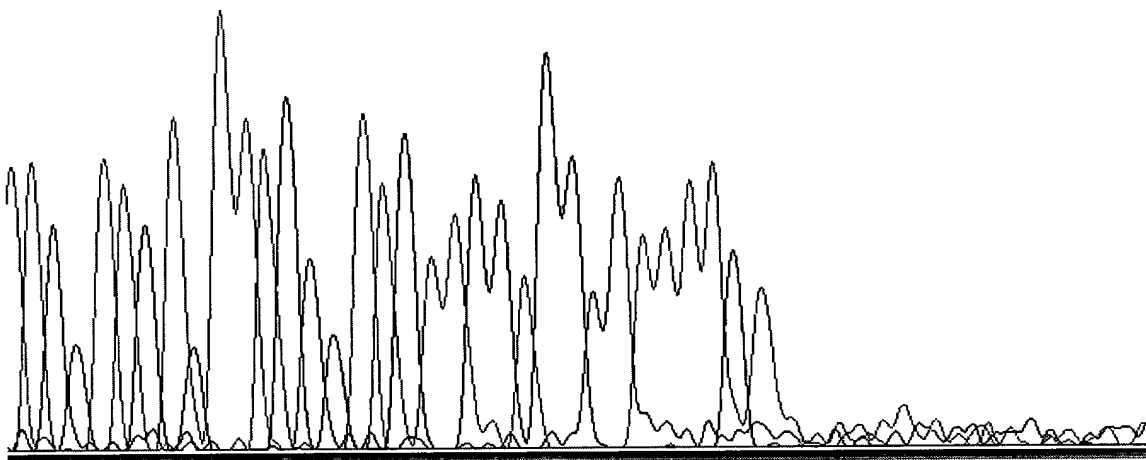
The primer is annealed and extended through the BtgZI site. When the reaction reaches the end of the molecule the Taq polymerase adds an extra A base.

5'-PRIMER-AGAATGCGATGTTGGTGGCCAAAAG(A)-3' (SEQ ID NO:3)

3'--..GCTACTCTTACGCTACAACCACCGGTTTTTC-5'

Sequencing Profile of BtgZI cut Sep-S3AI8.G10R DNA (ABI377 Sequencer)

1840 1920 2000 2080 2160 2240 2320 2400
ATCGATGAGAAATGCGATGTTGGTGGCCAAAAGAAANNTTTNCCTACCTNNCA
150 160 170 180 190



BtgZI Figure 2 : Determination of the BtgZI cleavage site.

Figure 2B: Location of cleavage on 5'-GCGATG-3' strand.

A DNA (Sep-S3AI8.G10L) having a BtgZI site and a convenient primer was cut with BtgZI, yielding ends indicated by the arrows:

5'--..TTCTTTCTGCGCGGTCAACT↓TTGTACCAATCATA**CATCGC**CTGAG..-3'
(SEQ ID NO:4)

3'--..AAGAAAGACGCGCCAGTTGAAACA↑TGGTTAGTAT**GTAGCG**GACTC..-5'

The resulting cleaved DNA:

5'--..TTCTTTCTGCGCGGTCAACT (SEQ ID NO:5)
(5'-TTGTACCAATCATA**CATCGC**CTGAG..-3')
(SEQ ID NO:6)

3'--..AAGAAAGACGCGCCAGTTGAAACA (3'- TGGTTAGTAT**GTAGCG**GACTC..-5')

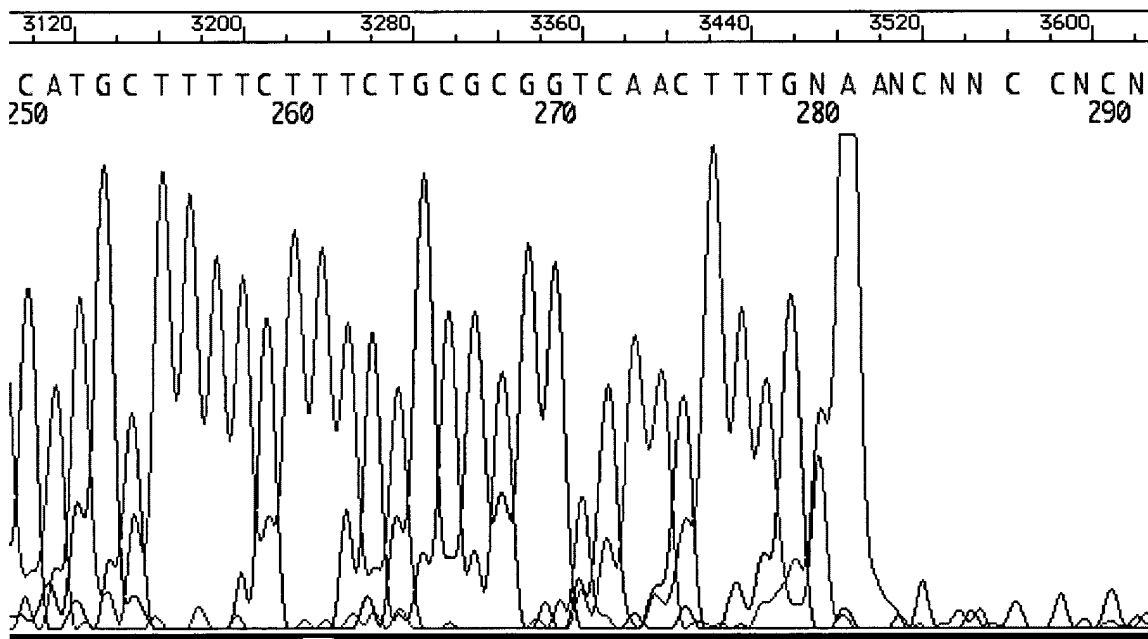
The template strand for dideoxy DNA sequencing extension:

3'--..AAGAAAGACGCGCCAGTTGAAACA

The primer is annealed and extended through the BtgZI site. When the reaction reaches the end of the molecule, the Taq polymerase adds an extra A base.

5'-PRIMER-> TTCTTTCTGCGCGGTCAACTTTGT(A)-3' (SEQ ID NO:7)

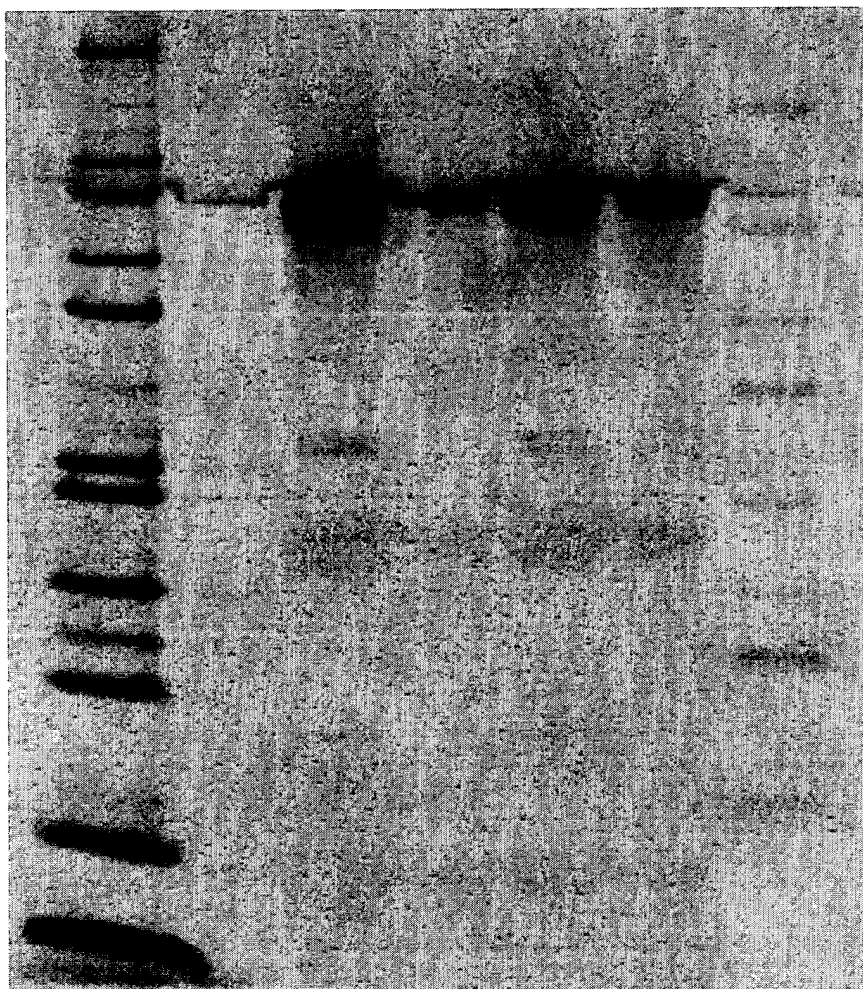
3'--.....AAGAAAGACGCGCCAGTTGAAACA - 5'



BtgZI Figure 3: SDS-PAGE gel of purified BtgZI endonuclease protein.

10 to 20% Tris-Tricine polyacrylamide gradient gel (Invitrogen-Novex),
stained with Coomassie blue R-250

1 2 3 4 5 6 7



BtgZI Figure 4

Amino-terminal amino acid sequence of the BtgZI endonuclease:

M Y W L L D Y V T Q Q K V R N D I N N L I K X I L X I